β-SBD-α-lid interfaces. We used pulsed dipolar ESR and ion mobility mass spectroscopy technologies to characterize the conformational ensembles of DnaK in its allosteric states to better understand how the nucleotide and substrates modulate the allosteric landscape. We found that DnaK has a narrow distance distribution in ATP-bound state, but broader distance distributions in all other states exhibit multiple coexisting components. The ATP+substrate ensemble reflects the tug-of-war between the forces of NBD-SBD interaction driven by the binding of nucleotides, and the force of β-SBD-α-lid interaction driven by the binding of substrates. The ATP+substrate state contains 24% of docked and 76% of undocked conformers. The ADP+substrate state has a smaller fraction of docked conformers and an additional species, which we may represent as a ''domain rotamer'' around the unbound linker. Rotation of the NBD and SBD around the interdomain linker may play an important role in the allosteric mechanism. The ATP+substrate state releases the SBD's helical lid from the NBD bound in the ATP-bound state to an SBD bound position and a ''free'' position; and the ADP+substrate state pushes the equilibrium from the free position to the SBD bound position. The allosteric states can be modulated by mutations to dissect the energetic contributions.

Platform: Excitation-Contraction Coupling

2539-Plat
Identification of a Calsequestrin-1 Mutation in a Human Vacuolar Myopathy
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Calsequestrin-1 (CASQ1) is the major calcium binding protein of the sarcoplasmic reticulum (SR) of skeletal muscle cells. It is mainly localized in the junctional domain of the SR where it is part of a quaternary complex, which includes the ryanodine receptor calcium release channel, junctin and triadin. Calsequestrin-1 can modulate Ca^{2+} release by either directly bind the ryanodine receptor and/or by binding to junctin and triadin. We recently identified a D244G mutation in CASQ1 in patients with a myopathy characterized by the presence of vacuoles containing aggregates of SR proteins. The mutation affects a conserved aspartic acid located in one of the high-affinity Ca^{2+} binding sites of CASQ1. We found that muscle fibers from patients carrying the CASQ1 mutation show alterations in the Ca^{2+} release kinetics, thus suggesting that the D244G mutation may alter the intracellular Ca^{2+} signaling in the affected fibers. Interestingly, mutations in the CASQ2 protein identified in patients affected by catecholaminergic polymorphic ventricular tachycardia (CPVT) were shown to alter either Ca^{2+} buffering or Ca^{2+} release properties of cardiac muscle cells and to reduce the ability of calsequestrin to bind junctin and triadin. In order to understand the cellular mechanisms responsible for alterations of Ca^{2+} release kinetics in skeletal muscle cells carrying the D244G mutation, interactions between mutated and wild type CASQ1 and the ryanodine receptor type 1, junctin and triadin are being investigated.

2540-Plat
Microtubule Detyrosination Modulates Stretch-Dependent X-ROS Signaling in Heart
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Mechano-chemo transduction, the conversion of mechanical signals into chemical ones, is a topic of accelerating interest in striated muscle. Axial stretch of a cardiac myocyte triggers an array of effects, including generation of reactive oxygen species and sensitization of the ryanodine receptor calcium release complex through a process termed X-ROS signaling. These phenomena are paralleled in skeletal muscle, and in both tissues are known to depend on the microtubule cytoskeleton. While past work has shown a microtubule dependence using drastic interventions, our current work focuses on specific patho/physiologically relevant posttranslational modifications of the microtubule cytoskeleton. We find that targeting the cleavage of a c-terminal tyrosine on alpha-tubulin (detyrosination) alters myocyte mechanical properties without disrupting overall microtubule structure. Inhibition of detyrosination significantly alters contractility and blunts mechanical effects on ROS production and calcium handling. We also find that detyrosinated microtubules are increased in Duchenne Muscular Dystrophy (DMD), and that inhibition of detyrosination alleviates dysfunctional calcium signaling and arrhythmias elicited by increased mechanical work in a DMD model. Taken together these results suggest a critical role for microtubule detyrosination in mechano-chemo transduction and identify a potential therapeutic target for the treatment of cardiomyopathy.

2541-Plat
Serca Located in the Junctional SR Shapes Calcium Release in Cardiac Myocytes
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Calcium-induced calcium release is in principle an all-or-nothing mechanism and self-propagation of Ca^{2+} waves is a pathological manifestation of this positive feedback loop. However, since local release of Ca^{2+} only elicits elementary Ca^{2+} release events (Ca^{2+} sparks) that do not develop into Ca^{2+} waves, Ca^{2+} release can be graded. This local control is possible because of the spatial arrangement of L-type Ca^{2+} channels and clusters of ryanodine receptors at the sites of Ca^{2+} release (dyads) (Stern 1992). We propose that the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) also contributes to local control by limiting diffusion of Ca^{2+}. Six days following conditional SERCA knockout, Ca^{2+} sparks exhibited broadened geometry and slowed kinetics (increase in spark width, time to peak, and duration by 33%, 33%, and 51%, respectively). At this time point, SERCA protein levels are reduced by 53% and SR Ca^{2+} content is decreased by 25% (Stokke et al. 2010). To determine the precise localization of SERCA we employed cryo immuno-gold electron microscopy on sections from the mouse papillary muscle. Normal cardiac myocytes showed preferential SERCA expression near the Z-lines (nearly twofold higher labeling density at the Z-line than the A-band) with SERCA molecules clearly located in the junctional SR in close proximity to the dyads. The conditional SERCA knockout resulted in preferential loss of labeling at these locations. From resin-embedded cross-sections of papillary muscles, we observed more abundant SR in the I-band compared to the A-band (by factor of 1.7 and 1.6 for control and KO respectively). These results suggest that SERCA contributes to local control of Ca^{2+} release by limiting diffusion of Ca^{2+} from the dyad. Thus, the processes of Ca^{2+} release and re-uptake are closely linked by a population of SERCA molecules in the junctional SR.

2542-Plat
Large Amplitude Rate-Dependent Mechanical Alternans may Precede Arrhythmogenesis in Human Heart Failure and are Linked to Electrical Alternans via Abnormal Calcium Handling
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Microvolt T-wave alternans (MTWA) testing identifies patients at risk for lethal ventricular arrhythmias. However, stratification of low and high risk patients with MTWA is challenging due in part to poor signal-to-noise ratio (SNR) of MTWA measurements. Since microscopic systolic pressure alternans (MSPA) has a higher SNR than MTWA, and is also associated with abnormal calcium handling, we hypothesized that rate-dependent MSPA also precedes arrhythmia and may be an alternative approach for arrhythmia risk stratification in heart failure patients. To test this hypothesis, we investigated mechanical alternans, a surrogate for MSPA, and its proposed link to arrhythmogenesis via abnormal calcium handling. Electromechanical models of single human myocytes were constructed. Key features of remodeling were incorporated to simulate abnormal calcium handling in human heart failure. A dynamical pacing protocol was used to investigate intracellular calcium concentration ([Ca]i), voltage, and force for different pacing rates. In normal myocytes, [Ca]i, voltage, and force alternans were not found for pacing rates <200 bpm. In the presence of deranged calcium handling common in heart failure (sarcoplasmic calcium reuptake reduced below 74%), [Ca]i, AP phase II voltage, and force alternans developed at moderate pacing rates <120 bpm and increased in magnitude with increased pacing rate. For all pacing rates, the ratio